METHODS OF USING IL-1 ANTAGONISTS TO TREAT NEOINTIMAL HYPERPLASIA

Cross-Reference to Related Applications

[0001] This application claims the benefit under 35 USC § 119(e) of U.S. Provisional 60/468,232 filed 6 May 2003, which application is herein specifically incorporated by reference in its entirety.

BACKGROUND

Field of the Invention

[0002] The invention relates to methods of using interleukin-1 (IL-1) antagonists to treat neointimal hyperplasia. In particular, the field of the invention is methods of treating restenosis and other neointimal hyperplasia conditions, including atherosclerosis, venous grafts of fistulae for hemodialysis, also known as vascular access dysfunction, bypass vein grafts, balloon angioplasty, hypertension, and related vascular diseases, using antagonists of IL-1-mediated biological activity.

Description of Related Art

[0003] Neointimal hyperplasia is the major complication associated with the progression of atherosclerotic plaques, chronic hypertension, and the injury response caused by surgical procedures treating vascular diseases, such as angioplasty and stenting. The activation of smooth muscle cells residing in the vessel wall stimulates their proliferation and migration into the intima area where they narrow the blood vessel and limit blood supply to the affected region. Surgical procedures to remove the neointima (endarterectomy) or to insert a solid support (stenting) have been the most effective treatments to correct the deficiency. Nonetheless, physical injury during these surgical procedures tends to accelerate neointima formation and cause the re-narrowing of the vessel, a process termed restenosis.

[0004] Tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1) are inflammatory cytokines that stimulate expression of adhesion molecules and induce the synthesis of other pro-inflammatory cytokines. TNFα and IL-1 are also known to influence vascular smooth muscle cell migration and

proliferation *in vitro*. Rectenwald et al. (2000) Circulation 102:1697-1702, have shown that TNFα and IL-1 modulate low shear stress-induced neointimal hyperplasia (NIH).

BRIEF SUMMARY OF THE INVENTION

[0005] In a first aspect, the invention features a method of treating, inhibiting, or ameliorating restenosis and other neointimal hyperplasia conditions, including atherosclerosis, vascular access dysfunction, hypertension and related vascular diseases, comprising administering to a subject in need an interleukin 1 (IL-1) antagonist. An IL-1 antagonist is a compound capable of blocking or inhibiting the biological action of IL-1, including fusion proteins capable of trapping IL-1, such as an IL-1 trap, interleukin-1 antagonist (IL-1ra), an anti-IL-1 antibody or fragment thereof, an anti-IL-1 receptor antibody or fragment thereof, a small molecule, or a nucleic acid capable of interfering with the expression of IL-1.

[0006] In a preferred embodiment, the IL-1 antagonist is an IL-1-specific fusion protein comprising two IL-1 receptor components and a multimerizing component, for example, an IL-1 trap described in U.S. patent publication No. 2003/0143697, published 31 July 2003, herein specifically incorporated by reference in its entirety. In a specific embodiment, the IL-1 trap is the fusion protein shown in SEQ ID NO:2, 4, 6, 8, 10, 12,14, 16, 18, 20, 22, 24, 26. A preferred IL-1 trap is shown in SEQ ID NO:10. In another embodiment, the IL-1 antagonist is an antibody or antibody fragment capable of binding IL-1α and/or IL-1β. In another embodiment, the IL-1 antagonist is an anti-IL-1 receptor (IL-1R1 or IL-1RAcp), or a fragment thereof. In specific embodiments, the IL-1 antagonist is a modified IL-1 trap comprising one or more receptor components and one or more immunoglobulin-derived components specific for IL-1 and/or an IL-1 receptor. In another embodiment, the IL-1 antagonist is a modified IL-1 trap comprising one or more immunoglobulin-derived components specific for IL-1 and/or an IL-1 receptor. In another embodiment, the IL-1 antagonist is IL-1ra (SEQ ID NO:27 (full-length molecule); SEQ ID NO:28 (mature protein). In yet another embodiment, the IL-1 antagonist is a nucleic acid capable of interfering with the expression of IL-1 . Examples of IL-1 antagonist nucleic acids include, for example, antisense molecules, inhibitory ribozymes designed to catalytically cleave gene mRNA transcripts encoding IL-1α, IL-1β, IL-1R1, IL-1RAcp, or short interfering RNA (siRNA) molecules.

[0007] The subject being treated is most preferably a human suffering from or at risk for the development of restenosis and other neointimal hyperplasia conditions, including atherosclerosis, vascular access dysfunction, hypertension and related vascular diseases.

[0008] The method of the invention includes administration of the IL-1 antagonist by any means known to the art, for example, subcutaneous, intramuscular , intranasal, intraarterial, intravenous, topical, transvaginal, transdermal, transanal administration or oral routes of administration.

[0009] In a second aspect, the invention features a method of preventing or inhibiting the development of restenosis in a subject in need thereof, comprising administering an IL-1 antagonist to a subject at risk for or suffering from restenosis, such that development of restenosis or the progression of the disease is inhibited.

[0010] In a third aspect, the invention features a method of preventing or inhibiting the development of atherosclerosis in a subject in need thereof, comprising administering an IL-1 antagonist to a subject at risk for or suffering from atherosclerosis, such that development of atherosclerosis or the progression of the disease is inhibited.

[0011] In a fourth aspect, the invention features a method of preventing or inhibiting the development of vascular access dysfunction in a subject in need thereof, comprising administering an IL-1 antagonist to a subject at risk for or suffering from vascular access dysfunction, such that development of vascular access dysfunction or the progression of the disease is inhibited.

[0012] In a fifth aspect, the invention features a method of inhibiting or ameliorating neointimal hyperplasia caused by hypertension and related vascular diseases, in a subject in need thereof, comprising administering an IL-1 antagonist to a subject at risk for or suffering from neointimal hyperplasia, such that development of neointimal hyperplasia or the progression of the disease is inhibited.

[0013] Accordingly, in a sixth aspect, the invention features pharmaceutical compositions comprising IL-1 antagonists with a pharmaceutically acceptable carrier. Such pharmaceutical compositions may comprise IL-1 traps or anti-IL-1 antibodies, antisense molecules, or siRNAs. The therapeutic methods of the invention may be treated with a combination of one or more IL-1 antagonists and a second therapeutic agent.

[0014] Other objects and advantages will become apparent from a review of the ensuing detailed

description.

Brief Description of the Figures

[0015] Fig. 1 shows the inhibitory effect of the IL-1 trap IL-1RAcp-IL-R1-Fc on neointima proliferation. IEL= internal elastic lamina; EEL= external elastic lamina.

[0016] Fig.2 compares wildtype and IL-1R knockout (KO) animals on neointima proliferation.

[0017] Figs. 3-4 shows the effect of APOE KO mice transfected with IL-1 trap (Fig. 3) or an Fc control and IL-1R2 trap (Fig. 4).

DETAILED DESCRIPTION

[0018] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only the appended claims.

[0019] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0020] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

General Description

[0021] The invention is based in part on the finding that administration of an agent capable of blocking or inhibiting IL-1-mediated biological activity is capable of decreasing , treating or preventing restenosis and other neointimal hyperplasia conditions, including atherosclerosis, vascular

access dysfunction, hypertension and related vascular diseases. Thus, the invention provides for methods of decreasing, treating or preventing restenosis and other neointimal hyperplasia conditions in a mammal by administering an IL-1 antagonist, in particular, an IL-1 trap, IL-1ra or anti-IL-1 antibodies.

Definitions

[0022] By the term "blocker", "inhibitor", or "antagonist" is meant a substance that retards or prevents a chemical or physiological reaction or response. Common blockers or inhibitors include but are not limited to antisense molecules, antibodies, antagonists and their derivatives. More specifically, an example of an IL-1 blocker or inhibitor is an IL-1 antagonist including, but not limited to, IL-1 trap.

[0023] By the term "therapeutically effective dose" is meant a dose that produces the desired effect for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

IL-1 Trap Antagonists

[0024] Interleukin-1 (IL-1) traps are multimers of fusion proteins containing IL-1 receptor components and a multimerizing component capable of interacting with the multimerizing component present in another fusion protein to form a higher order structure, such as a dimer. Cytokine traps are a novel extension of the receptor-Fc fusion concept in that they include two distinct receptor components that bind a single cytokine, resulting in the generation of antagonists with dramatically increased affinity over that offered by single component reagents. In fact, the cytokine traps that are described herein are among the most potent cytokine blockers ever described. Briefly, the cytokine traps called IL-1 traps are comprised of the extracellular domain of human IL-1R Type I (IL-1RI) or Type II (IL-1RII) followed by the extracellular domain of human IL-1 Accessory protein (IL-1AcP), followed by a multimerizing component. In a preferred embodiment, the multimerizing component is an immunoglobulin-derived domain, such as, for example, the Fc region of human IgG, including part of the hinge region, the CH2 and CH3 domains. Alternatively,

the IL-1 traps are comprised of the extracellular domain of human IL-1AcP, followed by the extracellular domain of human IL-1RI or IL-1RII, followed by a multimerizing component. For a more detailed description of the IL-1 traps, see WO 00/18932, which publication is herein specifically incorporated by reference in its entirety. Preferred IL-1 traps have the amino acid sequence shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26.

[0025] In specific embodiments, the IL-1 antagonist comprises an antibody fragment capable of binding IL-1α, IL-1β, IL-1R1 and/or IL-1RAcp, or a fragment thereof. One embodiment of an IL-1 antagonist comprising one or more antibody fragments, for example, single chain Fv (scFv), is described in U.S. 6,472,179, which publication is herein specifically incorporated by reference in its entirety. In all of the IL-1 antagonist embodiments comprising one or more antibody-derived components specific for IL-1 or an IL-1 receptor, the components may be arranged in a variety of configurations, e.g., a IL-1 receptor component(s) – scFv(s) – multimerizing component; IL-1 receptor component(s) – multimerizing component – scFv(s); scFv(s) – IL-1 receptor component(s) – multimerizing component, etc., so long as the molecule or multimer is capable of inhibiting the biological activity of IL-1. In another embodiment, the IL-1 antagonist is IL-1ra, including the full length protein of SEQ ID NO:27 or the mature protein of SEQ ID NO:28.

Anti-IL-1 Human Antibodies and Antibody Fragments

[0026] In another embodiment of the IL-1 antagonist useful in the method of the invention, examples of anti-IL-1 antibodies are disclosed in US 4,935,343; US 5,681,933; WO 95/01997; EP 0267611, US 6,419,944; WO 02/16436 and WO 01/53353. The IL-1 antagonist of the invention may include an antibody or antibody fragment specific for an IL-1 ligand (e.g., IL-1α or IL-1β) and/or an IL-1 receptor (e.g., IL-1R1 and/or IL-1RAcp). Antibody fragments include any fragment having the required target specificity, e.g. antibody fragments either produced by the modification of whole antibodies (e.g. enzymatic digestion), or those synthesized *de novo* using recombinant DNA methodologies (scFv, single domain antibodies or dAbs, single variable domain antibodies) or those identified using human phase display libraries (see, for example, McCafferty et al. (1990) Nature 348:552-554). Alternatively, antibodies can be isolated from mice producing human or human-mouse chimeric antibodies using standard immunization and antibody isolation methods, including but not

limited to making hybridomas, or using B cell screening technologies, such as SLAM. Immunoglobulin binding domains also include, but are not limited to, the variable regions of the heavy (V_H) or the light (V_L) chains of immunoglobulins.

[0027] The term "antibody" as used herein refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda , alpha, gamma, delta, epsilon, and mu constant regions, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. Within each IgG class, there are different isotypes (eg. IgG_1, IgG_2, IgG_3, IgG_4). Typically, the antigen-binding region of an antibody will be the most critical in determining specificity and affinity of binding.

[0028] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one light chain (about 25 kD) and one heavy chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100-110 or more amino acids primarily responsible for antigen recognition. The terms "variable light chain" (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0029] Antibodies exist as intact immunoglobulins, or as a number of well-characterized fragments produced by digestion with various peptidases. For example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region. While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology.

[0030] Methods for preparing antibodies are known to the art. See, for example, Kohler & Milstein (1975) Nature 256:495-497; Harlow & Lane (1988) Antibodies: a Laboratory Manual, Cold Spring

Harbor Lab., Cold Spring Harbor, NY). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Monoclonal antibodies can be humanized using standard cloning of the CDR regions into a human scaffold. Gene libraries encoding human heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity. Techniques for the production of single chain antibodies or recombinant antibodies (US 4,946,778; US 4,816,567) can be adapted to produce antibodies used in the fusion proteins and methods of the instant invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express human, human-mouse chimeric, or humanized antibodies. Alternatively, phage display technology can be used to identify human antibodies and heteromeric Fab fragments that specifically bind to selected antigens.

Antibody Screening and Selection

[0031] Screening and selection of preferred antibodies can be conducted by a variety of methods known to the art. Initial screening for the presence of monoclonal antibodies specific to a target antigen may be conducted through the use of ELISA-based methods, for example. A secondary screen is preferably conducted to identify and select a desired monoclonal antibody for use in construction of the multi-specific fusion proteins of the invention. Secondary screening may be conducted with any suitable method known to the art. One preferred method, termed "Biosensor Modification-Assisted Profiling" ("BiaMAP") is described in co-pending USSN 60/423,017 filed 01 Nov 2002, herein specifically incorporated by reference in its entirety. BiaMAP allows rapid identification of hybridoma clones producing monoclonal antibodies with desired characteristics. More specifically, monoclonal antibodies are sorted into distinct epitope-related groups based on evaluation of antibody:antigen interactions. Antibodies capable of blocking either a ligand or a receptor may be identified by a cell based assay, such as a luciferase assay utilizing a luciferase gene under the control of an NFKB driven promoter. Stimulation of the IL-1 receptors by IL-1 ligands leads to a signal through NFKB thus increasing luciferase levels in the cell. Blocking antibodies are identified as those antibodies that blocked IL-1 induction of luciferase activity.

Antisense Nucleic Acids

[0032] In a further embodiment, IL-1-mediated biological activity is blocked or inhibited by the use of IL-1 antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to the gene or cDNA encoding IL-1α, IL-1β, IL-1R1, or IL-1RAcp or portions thereof. As used herein, IL-1 "antisense" nucleic acids refers to nucleic acids capable of hybridizing by virtue of some sequence complementarity to a portion of an RNA (preferably mRNA) encoding IL-1α, IL-1β, IL-1R1, or IL-1RAcp. The antisense nucleic acids may be complementary to a coding and/or noncoding region of an mRNA encoding IL-1α, IL-1β, IL-1R1, or IL-1RAcp. Such antisense nucleic acids have utility as compounds that prevent IL-1α, IL-1β, IL-1R1, or IL-1RAcp expression, and can be used in the prevention or treatment of restenosis and other neointimal hyperplasia conditions, including atherosclerosis, vascular access dysfunction, hypertension and related vascular diseases. The antisense nucleic acids of the invention are double-stranded or single-stranded oligonucleotides, RNA or DNA or a modification or derivative thereof, and can be directly administered to a cell or produced intracellularly by transcription of exogenous, introduced sequences.

[0033] The invention further provides pharmaceutical compositions comprising a therapeutically effective amount of IL-1α, IL-1β, IL-1R1, or IL-1RAcp antisense nucleic acids, and a pharmaceutically acceptable carrier, vehicle or diluent.

[0034] The IL-1α, IL-1β, IL-1R1, or IL-1RAcp antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 50 oligonucleotides. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded.

Short interfering RNAs

[0035] In another embodiment, IL-1α, IL-1β, IL-1R1, or IL-1RAcp expression is inhibited by a short interfering RNA (siRNA) through RNA interference (RNAi) or post-transcriptional gene silencing (PTGS) (see, for example, Ketting et al. (2001) Genes Develop. 15:2654-2659). siRNA

molecules can target homologous mRNA molecules for destruction by cleaving the mRNA molecule within the region spanned by the siRNA molecule. Accordingly, siRNAs capable of targeting and cleaving homologous IL-1α, IL-1β, IL-1R1, or IL-1RAcp mRNA are useful for preventing or treating restenosis and other neointimal hyperplasia conditions, including atherosclerosis, vascular access dysfunction, hypertension and related vascular diseases.

Inhibitory Ribozymes

[0036] In another embodiment, restenosis and other neointimal hyperplasia conditions, including atherosclerosis, vascular access dysfunction, hypertension and related vascular diseases may be treated in a subject suffering from such diseases or disorders by decreasing the level of IL-1-mediated biological activity by using ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding IL-1α, IL-1β, IL-1R1, or IL-1RAcp, preventing translation of target gene mRNA and, therefore, expression of the gene product.

[0037] Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246. While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy mRNAs encoding IL-1α, IL-1β, IL-1R1, or IL-1RAcp, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA has the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art. The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA). The Cech-type ribozymes have an eight base pair active site that hybridizes to a target RNA sequence where after cleavage of the target RNA takes place. The invention encompasses those

Cech-type ribozymes that target eight base-pair active site sequences that are present in the genes encoding IL-1α, IL-1β, IL-1R1, or IL-1RAcp.

[0038] Standard methods for assessing cardiovascular health can be used to determine whether a subject is positively responding to treatment with the IL-1 antagonists. For example, after treatment with a IL-1 cytokine antagonist of the invention, a physician may choose to administer a stress test to determine that the subject is benefiting from administration of the cytokine antagonist. Generally the physician will monitor the patients activity level and general health to assess whether the subject is benefiting from administration of the cytokine antagonist. Thus, these as well as other methods known to the art, may be used to determine the extent to which the methods of the present invention are effective at treating neointimal hyperplasia including restenosis.

Treatment Population

[0039] Treatment population would include those people with vascular diseases being treated with coronary bypass surgery, angioplasty treatment, treatment with stents or drug coated stents; hemodialysis patients requiring grafts or fistulae; patients with elevated CRP levels with a history of vascular disease or atherosclerosis; patients diagnosed with peripheral vascular disease or hypertension; patients diagnosed with Buerger's disease, critical limb ischemia or thromboangiitis obliterans; patients undergoing vascular surgery for example, varicose veins, aneurysms, or arotic dissection.

Methods of Administration

[0040] The invention provides methods of treatment comprising administering to a subject an effective amount of an agent of the invention. In a preferred aspect, the agent is substantially purified (*e.g.*, substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, *e.g.,* such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0041] Various delivery systems are known and can be used to administer an agent of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol.

Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0042] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.*, by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, fibers, commercial skin substitutes or angioplasty balloons or stents.

[0043] In another embodiment, the active agent can be delivered in a vesicle, in particular a liposome (see Langer (1990) Science 249:1527-1533). In yet another embodiment, the active agent can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer (1990) *supra*). In another embodiment, polymeric materials can be used (see Howard et al. (1989) J. Neurosurg. 71:105). In another embodiment where the active agent of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see, for example, U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al.,

1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

Cellular Transfection and Gene Therapy

[0044] The present invention encompasses the use of nucleic acids encoding the IL-1-specific fusion proteins of the invention for transfection of cells *in vitro* and *in vivo*. These nucleic acids can be inserted into any of a number of well-known vectors for transfection of target cells and organisms. The nucleic acids are transfected into cells *ex vivo* and *in vivo*, through the interaction of the vector and the target cell. Reintroduction of transfected cells may be accomplished by any method known to the art, including re-implantation of encapsulated cells. The compositions are administered (e.g., by injection into a muscle) to a subject in an amount sufficient to elicit a therapeutic response. An amount adequate to accomplish this is defined as "a therapeutically effective dose or amount."

[0045] In another aspect, the invention provides a method of treating or preventing neointimal hyperplasia in a human comprising transfecting a cell with a nucleic acid encoding an IL-1-specific fusion protein of the invention, antibody or IL-1ra, wherein the nucleic acid comprises an inducible promoter operably linked to the nucleic acid encoding the IL-1-specific fusion protein antibody or IL-1ra. For gene therapy procedures in the treatment or prevention of human disease, see for example, Van Brunt (1998) Biotechnology 6:1149-1154.

Combination Therapies

[0046] In numerous embodiments, the IL-1 antagonists of the present invention may be administered in combination with one or more additional compounds or therapies or surgical procedures. For example, a suitable therapeutic agent for use in combination, either alternating or simultaneously, with the IL-1 antagonists may include anti-platelet therapy such as aspirin, Reopro™ (Lilly), anti-p-selectin antibodies; antithrombolic and blood thinning agents, such as Retavse™ (Centocor); Streptase™ (AstraZeneca), TNKase™ (Genentech), Ticlid™ (Roche) and Plavix™ (Bristol-Myers Squibb) and heparin; HMG-CoA reductase inhibitors, such as Baycol™ (Bayer), Lescol™ (Noavartis), Lipitor™ (Pfizer), Mevacor™ (Merck), Pravachol™ (Bristol Myers Squibb, Zocor™ (Merck) or antilipidemic agents such as, Colestid™ (Pfizer), WelChol™ (Sankyo),

Atromid-S™ (Wyeth), Lopid™ (Pfizer), Tricor™ (Abbott); agents effective to treat or prevent restenosis such as Sirolimus ™ (Wyeth, Johnson & Johnson), dexamethasone (Merck), Predisolone™ (Muro, Mylan, Watson, We), Tacrolimus™ (Fujisawa), Pimecrolimus ™ (Novartis) Taxol/Paclitaxel (Bristol-Myers Squibb), or Methotrexate (Baxter, Mylan, Roxane) ; anti-fibrolytic agents such as antibodies against TGFß PDGF, or CTGF; PDGF inhibitors such as Gleevec™ (Novartis); anti-inflammatory agents such as antibodies, peptides and other inhibitors of CD11a/CD8 (Mac1) [Raptiva™ (Genentech)], ICAM, C5a and TNFa [Humira™ (Abbott), Enbrel™ (Amgen), Remicade™ (Centocor)], Thalidomide™ (Celltech); hypertension drugs, such as ACE inhibitors [Accupril™ (Parke-Davis); Altace™ (Monarch); Captopril™ (Mylan); Enalaprilate™ (Baxter); Lotensin™ (Novartis); Mavik™ (Bristol-Myers Squibb); Prinivil™ (Merck); Univasc™ (Schwarz), Vasotec™ (Merck)]. In addition the IL-1 antagonists may be used in combination, either alternating or simultaneously, with surgical procedures including but not limited to surgical stenting and balloon angioplasty.

Pharmaceutical Compositions

[0047] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of an active agent, and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional

binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0048] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0049] The active agents of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0050] The amount of the active agent of the invention which will be effective in the treatment of delayed-type hypersensitivity can be determined by standard clinical techniques based on the present description. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20 micrograms to 2 grams of active compound per kilogram body weight. Suitable dosage ranges for intra-nasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0051] For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used

to more accurately determine useful doses in humans. Initial dosages can also be estimated from *in vivo* data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

[0052] Dosage amount and interval may be adjusted individually to provide plasma levels of the compounds that are sufficient to maintain therapeutic effect. In cases of local administration or selective uptake, the effective local concentration of the compounds may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

[0053] The amount of compound administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician. The therapy may be repeated intermittently while symptoms are detectable or even when they are not detectable. The therapy may be provided alone or in combination with other drugs.

Kits

[0054] The invention also provides an article of manufacturing comprising packaging material and a pharmaceutical agent contained within the packaging material, wherein the pharmaceutical agent comprises at least one IL-1-specific fusion protein of the invention and wherein the packaging material comprises a label or package insert which indicates that the IL-1-specific fusion protein can be used for treating neointimal hyperplasia.

[0055] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

[0056] The following example is put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Experimental protocol and surgical procedures

[0057] Animals: C57Bl6 mice, interleukin-1 receptor type I deficient (IL-1RI -/-) mice and apolipoprotein E deficient (APOE -/-) mice at the age of 8 to 12 weeks were used in this study to assess vascular response to injury. Both of the genetically modified mice are congenic to the C57Bl6 background. All animals were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed in individual cages after surgery and were allowed *ad libitum* access to regular chow and water.

[0058] Vascular injury model: Vascular injury was induced by surgical ligation of the left common carotid artery before bifurcation. Mice were anesthetized by intraperitoneal injection of a Ketamine/xylazine solution. After anesthesia was attained, a midline incision was made at the tracheal area from the caudal end of the larynx to the suprasternal notch. After separating the sternothyroideus muscle on the left side, the pulsating carotid artery was identified and ligated with sterile 6-0 silk sutures. The skin was closed with sterile sutures.

[0059] Delivery of murine IL-1 trap: Expression of murine IL-1 trap was introduced using a hydrodynamics-based administration of plasmid DNA via tail vein injection. Four days prior to surgery, animals were divided equally into two groups. One group of mice received 50-100 μg/animal plasmid DNA carrying the murine IL-1 trap expression vector. The other group of mice received an equal amount of empty control vector DNA. Mice were anesthetized using Isoflurane™. Plasmid DNA diluted in sterile saline in an amount equivalent to 10% (V/W) of the mouse body weight was injected promptly into tail vein.

[0060] Tissue harvesting and preparation: Twenty-eight days after carotid artery ligation, the mice were anesthetized and whole blood was collected from each mouse through cardiac puncture. Subsequently, mice were perfused with saline followed by 10% neutral buffered formalin through the left cardiac ventricle. The entire neck section was collected from each mouse and post-fixed in 10%

formalin for an additional 48 hours. Fixed neck tissue was decalcified in 10% EDTA solution for 8 days with frequent changes of fresh solution. Decalcified neck tissue were embedded in gelatin by sequential incubation in 5%, 10% and 25% gelatin solution and prepared for cryosection. Ligation sites were identified by suture position. Seven successive 10 mm sections at 500 mm intervals proximal to the ligation site were used for morphometric analysis per mouse.

[0061] Morphometry: Cryosections were stained with standard hematoxylin and eosin methodology. The images of injured arteries and contra-lateral uninjured arteries were captured using a Nikon Microphot microscope and SPOT software and a SPOT RT COLOR camera. The length encircling the lumen, the internal elastic lamina (IEL) and the external elastic lamina (EEL) was determined by digitally tracing the perimeter of each layer using Bersoft Image Measurement 2.01 software. The length was then converted to circumference assuming the native artery formed a circular structure to calculate the area. The neointima area was defined as the difference between the areas of lumen and IEL. The thickness of media was calculated as the difference of radius between IEL and EEL.

[0062] Results. A mouse vascular injury model was used to investigate factors mediating the formation of neointima. The left common carotid artery of C57Bl/6 mouse was surgically ligated at the position before the bifurcation. The occlusion of the common carotid artery stimulates significant neointima formation proximal to the ligation site over a four week period. Interleukin-1b appears to play an important role in this response because (1) the expression of interleukin-1b is substantially increased under the injury condition and (2) the neointima formation is suppressed in a genetically engineered mouse lacking the signal transducing receptor of interleukin-1 (IL-1RI knockout mouse) (Rectenwald (2000) Circulation 102:1967-1702). However, IL-1α may also play a role, thus the IL-1 traps are a superior method of blocking the resposne over those that block only a single IL-1 ligand.

[0063] To examine if the interleukin-1 antagonist IL-1 trap can inhibit neointima formation, IL-1 trap IL-1RAcp-IL-1R1-Fc trap expressed *in vivo* using a hydrodynamic-based transfection method with plasmid DNA vector four days prior to the surgery. Half of C57Bl/6 mice (N=10) received plasmid DNA carrying an IL-1 trap expression vectors. Others were injected with empty control vector DNA. Four weeks after surgical injury, mice were sacrificed for subsequent analyses.

Histological examination of the injured vessels revealed that the neointima formation was completely blocked in IL-1 trap-expressing animals, whereas the arteries from the vector control group revealed a significant accumulation of cells under the endothelial cell layer. Morphometric quantitation of the common carotid arteries over a 3.5 mm length demonstrated the inhibitory effect of the IL-1Racp-IL-1R1-Fc trap in neointima proliferation (Fig. 1). The neointimal growth is determined as the area between the IEL, and the lumen and thus is the difference between the IEL and Lumen radii. The effect of the lack of IL-1R1 in knockout mice is shown in Fig. 2. Similar reductions on neointimal growth were seen in the trap-treated mice as were seen in the IL-1R1 knockout indicating the IL-1 trap has an effect similar to a complete blockade of IL-1 signaling.

[0064] APOE is a surface protein of serum lipoprotein particles whose deficiency results in hypercholesterolemia and spontaneous atherosclerotic plaque formation in APOE knockout mice. APOE deficiency also exacerbates arterial injury causing increased neointima formation with necrotic cores similar to advanced fibroatheroma. To test if the IL-1 trap could prevent the neointima formation under APOE-deficient conditions, APOE knockout mice were transfected with either an IL-1RAcp-IL-1R1-Fc or IL-1Acrp-IL-1R2-Fc trap expression vector or an Fc control vector before surgical injury. At 28 days after surgical injury, it was found that the IL-1 trap treatments caused a 85% reduction of neointima area including the fibrous cap (Figs. 3 and 4). This confirms that the IL-1 Traps are potent blockers of neointima formation under hypercholesterolemia condition.